

PROJECT NUMBER: 1902
PROJECT TITLE: Cell/Tissue Culture Research
PROJECT LEADER: D. Teng
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PERIOD COVERED: May, 1990

I. TOBACCO MICROBIOLOGY

A. Objective: To develop methods and to evaluate the microflora resident in tobacco materials.

B. Results:

1. Bright Casing Storage Study

Samples of bright casing stored at 4, 27, 37.5 and 50° C have been analyzed for changes in microbial populations after 0, 24 hours, 48 hours, 1 week and 2 weeks of storage. To date, no detectable microbial population has been observed in any of the analyzed samples (1).

2. Enumeration of Bacteria

In order to investigate alternative methods for the reliable determination of bacterial numbers associated with tobacco, the Most Probable Number (MPN) method via the use of the Vitek Bioburden Enumeration Card was compared to our standard operating procedure (SOP) for viable plate counts (2). The bacterial counts from DBC burley tobacco obtained with the two methods were not comparable in 3 experiments with 3 replicates per experiment (3).

3. Determination of Ergosterol

The FTR procedure for the determination of ergosterol (4) was evaluated as an alternative indicator of mold growth in tobacco. The HPLC standard curve for ergosterol produced excellent linearity ($R^2 > 0.99$) in the 1 - 50 $\mu\text{g/ml}$ range (5). A peak from DBC bright tobacco with an elution time of 46.5 minutes could not be eliminated with the silica gel cleanup recommended. Its presence lengthens the HPLC time considerably since ergosterol elutes at 8.6 minutes.

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C. Plans:

1. Continue the Bright Casing storage study and perform analyses after 1, 2, 3 and 4 months.
2. Issue a memo reporting the findings regarding the enumeration of bacteria via MPN.
3. Attempt to eliminate the 46.5 minute peak present in HPLC determinations of ergosterol.

D. References:

1. Chadick, D. Notebook No. 8825, p. 156.
2. Crockett, E. Special Report Number 86-055, 1986 February 20.
3. Gaines, O. Notebook No. 8904, pp. 67-70, 76-98.
4. Bindler, G. N., Piadé, J. J. and Schulthess, D. Evaluation of selected steroids as chemical markers of past or presently occurring fungal infections on tobacco. *Beitrage zur tabakforschung*. 14(2): 127-134; 1988 October.
5. Weissbecker, L. Notebook No. 8822, p. 127.

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